



NAVAL RESEARCH
ADVISORY COMMITTEE

ARTIFICIAL
BLOOD

REQUIREMENTS FOR DELIVERY OF "ARTIFICIAL BLOOD" TO THE MILITARY

Report Documentation Page				Form Approved OMB No. 0704-0188	
Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.					
1. REPORT DATE AUG 1992		2. REPORT TYPE		3. DATES COVERED 00-00-1992 to 00-00-1992	
4. TITLE AND SUBTITLE Requirements for Delivery of 'Artificial Blood' to the Military				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Naval Research Advisory Committee, 875 North Randolph Street Suite 1230, Arlington, VA, 22203-1995				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Same as Report (SAR)	18. NUMBER OF PAGES 92	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified			

TABLE OF CONTENTS

SECTION	TITLE	PAGE
I.	EXECUTIVE SUMMARY	5
II.	OPENING STATEMENT	9
III.	INTRODUCTION	11
	CONGRESSIONAL MANDATE	13
	CONGRESSIONAL TASKING	17
	TERMS OF REFERENCE	19
	PANEL MEMBERSHIP	21
	PRESENTATIONS	23
IV.	BACKGROUND	25
V.	TECHNOLOGY OVERVIEW	41
VI.	DEVELOPMENT OF RED CELL SUBSTITUTES	63
VII.	CONCLUSIONS AND RECOMMENDATIONS	75
VIII.	APPENDIX A - GLOSSARY	85
IX.	APPENDIX B - ADDITIONAL RESOURCE MATERIAL	87
X.	APPENDIX C - DEPT OF HEALTH AND HUMAN SERVICES LETTER OF 11 MARCH 1992 TO OCNR	89

EXECUTIVE SUMMARY

The Committees on Armed Services of the 102nd Congress directed the Secretary of the Navy, on behalf of the Department of Defense, to report on current technology and prospects for development of a safe, effective, commercially produced blood substitute approved for clinical use. Recommendations to accelerate availability of such a substitute to the military and the Nation were also sought. The Assistant Secretary of the Navy (Research, Development and Acquisition) directed the Chairman of the Naval Research Advisory Committee (NRAC) to convene a panel to address these issues.

The House of Representatives Conferees Report directed the Department of Defense to provide an assessment of all “promising emerging technologies and ... assess each technology’s potential to satisfy civilian artificial blood supply needs....” The tasking specified that this assessment be fully coordinated with the National Institutes of Health and “any other public or private sector activity involved in artificial blood substitute development.”

The NRAC Panel was specifically designed to comply with the Congressional guidance and included representation from relevant technology areas of industry, academia and government. The Panel Membership consisted of internationally recognized experts in their fields from the National Institutes of Health, the Food and Drug Administration, the Office of the Secretary of Defense, colleges and universities, and industry. This group included active and retired officers from the Medical Corps and Medical Service Corps of the U.S. Navy, U.S. Army, and U.S. Public Health Service.

The Panel met over a five month period (March - July 1992). Because of the intensely competitive nature of this industrial field, all meetings were held in closed session. Panel members were screened for conflicts of interest, approved by the Office of the Secretary of Defense, and appointed as Special Government Employees (SGE) if not already full-time government employees. All Panel members signed “non-disclosure agreements” before access was provided to any proprietary information. Because of the importance of this project, the Navy obtained authorization from the Food and Drug Administration (FDA) for Panel members from the FDA to discuss in closed-session “FDA information regarding artificial blood otherwise exempt from disclosure.” This authorization did not include “trade secret information prohibited from disclosure by statute.” (Appendix C.)

After consideration of important issues for this study, Panel members reviewed information submitted in response to both an announcement published in the Commerce Business Daily and to individual solicitation. Information was provided in written submissions and by individual closed-session presentation to the Panel. The extensive discussion and careful deliberation that followed resulted in the observations, conclusions, and recommendations presented in this report.

Although the Nation’s blood supply is safer than it has ever been, the potential to improve logistical efficiency in supplementation of oxygen carrying capacity, reduce or eliminate risk of disease transmission, and reduce cost has far reaching beneficial

implications. The Panel believes that a safe, effective, and practical red cell substitute is feasible, and this difficult goal should be actively pursued until achieved. To do this most effectively, a National commitment is required. Greater understanding of basic mechanisms and toxicities is needed. Lack of this knowledge will likely preclude availability of an approved product in the near future.

The needs of the military for a red cell substitute were considered in comparison to requirements for a product for civilian use. While the military may have greater concerns for logistical considerations (e.g. weight, volume, storage temperature), these concerns are relative. When used for the same clinical indication, there are no unique military requirements for an “ideal” substitute that differ significantly from an “ideal” product for civilian use. Even when an acceptable substitute is available, a blood banking system will still be needed to supply other elements of whole blood required for specific purposes other than carrying oxygen.

All technologies reviewed seek to approach the exquisite balance of structure and function found in the normal red cell. Hemoglobin free in the bloodstream (outside the red cell) carries oxygen, but it rapidly breaks down into its subunits and exerts many adverse effects. Some technologies are directed at overcoming problems associated with free hemoglobin. An example is chemical modification of human or non-human (mostly bovine) hemoglobin in an effort to retain the beneficial oxygen carrying properties of the free hemoglobin while minimizing deleterious effects. Other technologies use genetic engineering for production and modification of hemoglobin generated in bacteria, yeast, or other animal species. Hemoglobins may also be encapsulated in artificial cell membranes to simulate the red cell. A technology not based on hemoglobin involves the use of emulsions of perfluorocarbon compounds which carry oxygen in the blood dissolved (not bound) in the emulsion.

Industry has built upon information generated by previous publicly funded research in the field. Industry is developing facilities to produce purified products, and testing in animals and preliminary tests in humans are in progress. Much proprietary research has been conducted by industry, but because of its proprietary nature, the information often does not benefit from peer-review and scrutiny in the scientific literature. Furthermore, it is not often made available to expand the scientific base of knowledge in the field.

In early clinical trials in humans (Phase I) reported to the FDA, small doses of product were given to normal healthy volunteers. Phase I testing using soluble hemoglobins and perfluorocarbon emulsions has been done. Results have shown some adverse effects for each of the products tested. No product has been allowed to progress to Phase II testing. Surprisingly, adverse reactions reported in Phase I trials in humans were not predicted by results of prior animal studies. The complexity of problems associated with development of a red cell substitute will likely preclude its availability for several years.

The field is not sufficiently advanced to identify a leading technology, and progress is stalled. Problems are not likely to be solved in the near future without a National commitment to developing a red cell substitute. This red cell substitute

program should be funded for a period of at least five years (beginning in FY 93) at a level of \$50 million per year. This funding should not be to the exclusion of other blood related research. Funding for product development and manufacturing should continue to come from the private sector.

The Panel recommends that the Congress direct the Department of Defense and National Institutes of Health, with the active support of the Food and Drug Administration, to work together to jointly establish and execute a program of excellence based on peer-review and scientific merit. Critical obstacles in development of a red cell substitute must be addressed and overcome. This effort should foster an increase in the number of investigators in the field. It is essential that all information generated by this program be disseminated in the scientific literature in a timely manner, or otherwise remain in the public domain.

Successful development of an approved red cell substitute will likely have many beneficial implications beyond the scope of those currently envisioned.



**The Nation would benefit from a safe, effective,
and practical red cell substitute. Without a major
national commitment to relevant research,
prospects for its development within the next
five years are unlikely.**

In excess of \$2 billion per year are spent on maintaining the Nation's blood supply. The Nation's blood supply, for both military and civilian use, is safer than it has ever been. Blood for transfusion is obtained from volunteers who are screened for various transmissible agents of infectious diseases, such as hepatitis viruses or human immunodeficiency viruses (HIV). These tests are highly sensitive and specific. On rare occasions, however, a unit of contaminated blood escapes detection. Donated red blood cells are refrigerated for up to 42 days and then discarded. To ensure that red blood cells are compatible with the blood of the intended recipient, cross-matching is required. For bleeding patients in urgent need of transfusions, the time required to process stored blood can affect chances for survival of the patient.

Development of an artificial red blood cell substitute that is free of transmissible agents of infectious diseases, stable at room temperature for long periods of time, universally compatible, and immediately transfusable is a formidable challenge. A number of pharmaceutical and biotechnology companies are actively working on development of an acceptable product. However, a product capable of meeting requirements for regulatory approval is not readily identifiable. Our best estimate is that five or more years of intensive effort will be required to develop a safe red cell substitute.

III. INTRODUCTION



- **HOUSE COMMITTEE ON ARMED SERVICES**
 - **H.R. 102-60**
- **SENATE COMMITTEE ON ARMED SERVICES**
 - **S.R. 102-113**
- **HOUSE AND SENATE CONFERENCE REPORT
TO ACCOMPANY H.R. 2100 (H.R. 102-311)**

Language from the National Defense Authorization Act for Fiscal Years 1992 and 1993, Report of the Committee on Armed Services, House of Representatives (H.R. 102-60) follows:

“Artificial Blood Substitutes

The committee recommends continued research into blood substitutes and developmental technology that decreases the need for large supplies of whole human blood stores and the costly logistics associated with storage, cross matching, shelf life inventory, donor screening and collection. The committee recognizes the advancements made in recombinant human hemoglobin (RHH) and the potential this product holds for the military forces as well as implications for solutions to some world health problems.

The committee directs the Secretary of the Navy to assess opportunities to accelerate product availability through testing, development of manufacturing methods or other developmental support where warranted for implementation into the military. Support for RHH

must be assessed against other blood substitutes technology considering early availability. The assessment shall contain a strategy and plan that identifies a development framework, timetable, and investment recommendation and shall be delivered to the Committees on Armed Services of the Senate and the House of Representatives by March 1, 1992"

Language from the National Defense Authorization Act for Fiscal Years 1992 and 1993, Committee on Armed Services, United States Senate (S.R. 102-113) follows:

"Artificial Blood Substitutes

The committee believes that new developments in the field of hemoglobin-based red blood cell substitutes may offer substantial benefits to the military in both peacetime and combat. Cost factors and logistical problems associated with the sourcing, screening, testing, typing, cross-matching, shipping and storage of blood would be substantially reduced. The need for frequent replacement of large inventories would also be reduced or eliminated. Disruption of the civilian blood supply and other problems stemming from collecting blood during rapid mobilization could also be largely avoided. In addition, recent advancements in the area of recombinant human hemoglobin (RHH) show promise of providing a product that may offer new capabilities for far-forward casualty care as well as totally eliminating any risk associated with blood-borne infectious agents in operating room transfusions.

The committee recognizes the unique benefits to the military of rapid development of this vital technology. The committee encourages the Defense Department to provide specific assistance to accelerate product development. The Department of Defense should report its progress in supporting this technology to the Committee on Armed Services of the Senate and the House of Representatives by March 1, 1992."

Language from the National Defense Authorization Act for Fiscal Years 1992 and 1993, Conference Report to accompany H.R. 2100 follows:

"Artificial Blood Substitutes

The House report (H. Rept. 102-60) directed the Secretary of the Navy to assess opportunities to accelerate the availability of recombinant human hemoglobin (RHH) derived artificial blood substitutes. Support for RHH must be assessed against other blood technology in considering

accelerating its early availability. A plan describing the development framework, timetable, and investment recommendation was directed to be provided to the Committees on Armed Services of the Senate and House of Representatives by March 1, 1992.

The Senate report (S Rept. 102-113) contained similar direction.

The conferees understand that RHH represents only one of many emerging approaches in developing artificial blood substitutes. The conferees do not intend that the Defense Department plan for accelerating product availability of an artificial blood substitute concentrate on the RHH technology exclusively. The Department of Defense should, instead, look without prejudice at all promising emerging technologies and prepare a plan for those technologies that best fits the Department's needs. The conferees hope that the Department would also assess each such technology's potential to satisfy civilian artificial blood supply needs, and to eventually become commercially self-supporting. In this regard, the conferees believe that the Department's assessment should be fully coordinated with the National Institute of Health and any other public or private sector activity involved in artificial blood substitute development.

To ensure that adequate time exists to fully consider all alternatives, the conferees agree to delay the submission date for the artificial blood substitute plan until July 30, 1992."



- **Look at all Promising Technologies**
- **Prepare a Plan That Best Fits DoD Needs**
- **Assess Technology's Potential to Satisfy Civilian Needs and Become Commercially Self-Supporting**
- **Coordinate with NIH and any Other Public or Private Sector Activity Involved**

This is a summary of the language from the conferees report from the National Defense Authorization Act for Fiscal Years 1992 and 1993, Conference Report to accompany H.R. 2100.



TERMS OF REFERENCE GENERAL OBJECTIVE

ARTIFICIAL
BLOOD

Determine opportunities to accelerate the availability of an effective, safe, commercially produced and licensed blood (red cell) substitute to the United States military.

The NRAC Terms of Reference (TOR) that was developed from congressional tasking is as follows:

GENERAL OBJECTIVE

To evaluate opportunities to accelerate availability of artificial blood substitute products, (oxygen carrying), through testing, development of manufacturing methods or other developmental support where warranted for military use and other future applications; and to develop a strategy and plan that identifies a development framework, timetable, and investment recommendation for Congress.

BACKGROUND

HASC Report 102-60 of 13 May 1991 directed the Secretary of the Navy to provide the report outlined above. "Artificial Blood," when developed, will have such obviously important and far reaching effects for the military and the world in general as to be compared to the development of blood transfusion technology earlier in this century. A "Sources Sought" announcement has been published in the Commerce Business Daily of 29 August 1991 to assess interest in participating in this process. Eighteen organizations, as well as the Department of Defense and U.S. Army, have expressed interest.

SPECIFIC TASKING

- a. Determine the opportunities for acceleration of artificial blood substitute products into the military by evaluating the state of testing, state of manufacturing methods, appropriate accomplishments of non-DoD investments, and developmental support requirements for implementation.
- b. Make recommendations for a strategy and plan that consider developmental framework, implementation timetable, and investment strategy for the military.
- c. Make all recommendations and assessments necessary to result in an effective, safe, commercially produced, and licensed erythrocyte (oxygen carrying) substitute being made available to the United States military.

PANEL MEMBERSHIP

CHAIRMAN

William Neal, M.D.
Professor and Chairman,
Dept. of Pediatrics, West Virginia University

George Biro, M.D., Ph.D.
Professor of Physiology
Faculty of Medicine
University of Ottawa
Canada

John Collins, M.D.
Professor of Surgery
Stanford University

Joseph C. Fratantoni, M.D.
Chief, Laboratory of Cellular Hematology
Office of Biologics Research, Center
for Biologics Evaluation and Research
U. S. Food and Drug Administration

Kenneth S. Landreth, Ph.D.
Professor, Department of Microbiology
and Immunology and Member,
Mary Babb Randolph Cancer Center
West Virginia University

George J. Nemo, Ph.D.
Chief, Transfusion Medicine Branch
Division of Blood Diseases and Resources
National Heart, Lung and Blood Institute

C. Robert Valeri, M.D.
Director
Naval Blood Research Laboratory
Boston University School of Medicine

ASN(RD&A) SPONSOR

James J. DeCorpo, Ph.D.
Director, Office of Advanced Technology
Office of the Chief of Naval Research

VICE CHAIRMAN

Dennie M. Welsh
President and CEO
Integrated Systems Solutions Corp.

Joy A. Cavagnaro, Ph.D.
Asst. Director, Pharmacology and
Toxicology, Office of Biologics
Research, Center for Biologics
Evaluation and Research
U. S. Food and Drug Administration

M. A. Cramer, Jr.
President and CEO
Vitro Corporation

Robert B. Jennings, M.D.
James B. Duke Professor of Pathology
Duke University Medical Center

Walker Long, M.D.
Director, Cardio-Pulmonary Medicine
Burroughs Wellcome Company and
Research Associate Professor
University of North Carolina at
Chapel Hill

CDR David A. Reichman, MSC, USN
Ph.D., MT (ASCP) SBB
Deputy Director for Operations
Armed Services Blood Program Office

Robert M. Winslow, M.D.
Adjunct Professor
University of California, San Diego

EXECUTIVE SECRETARY

CDR Fred P. Paleologo, MC, USNR
Naval Medical Research and
Development Command

ASSOCIATE EXECUTIVE SECRETARY

LT Christine M. Grabowski, MSC, USNR

INDUSTRY & DoD PRESENTATIONS AND OTHER SOURCES OF INFORMATION

INDUSTRY PRESENTATIONS:

Alliance Pharmaceutical Corp.	Baxter Healthcare Corp.
BIOPURE Corp.	BioTime Inc.
Cerny Land of Utica	Cryopharm
DNX	FLS Acquisition Corp. (dba Biokinetics)
LifeCell Corp.	Somatogen, Inc.
Strotech, Inc.	

DoD PRESENTATIONS:

Deputy Assistant Secretary of Defense (Health Affairs)
Office of Advanced Technology, OCNR
Naval Medical Research and Development Command
Armed Services Blood Program Office
Naval Research Laboratory
Letterman Army Institute of Research
U.S. Army Medical Research and Development Command

INDIVIDUAL PRESENTATIONS:

Rudolph Garcia-Gallont, M.D. University Francisco Marroquin Medical School Herrera-Llerandi Hospital Guatamala City	Herbert Wallace, M.D. and Roger Schnaare, Ph.D. The Graduate Hospital Philadelphia
Gerald L. Pollack, Ph.D. Michigan State University East Lansing	Timothy M. Townes, Ph.D. University of Alabama Birmingham
Richard L. Beissinger, D.E.S. and David L. McCormick, Ph.D. IIT Research Institute Chicago	

WRITTEN INFORMATION ONLY:

Bio-Molecular Works International

Leland C. Clark, Jr., Ph.D.
University of Cincinnati

Liposome Technology, Inc.

Prof. Dr. Eishun Tsuchida
Waseda University
Tokyo

Northfield Laboratories

The Shaw Group (HemaGen
PFC Ltd.)

Vestar, Inc.

IV. BACKGROUND



WHY DEVELOP A RED CELL SUBSTITUTE?

ARTIFICIAL
BLOOD

- **Eliminate Disease Transmission**
- **Improve Availability**
- **Eliminate Incompatibility**
- **Reduce Cost**
- **Simplify Use**

An ideal red cell substitute would eliminate disease transmission and the need for cross-matching, and would simplify transfusion practice. An acceptable blood substitute should also minimize problems of availability associated with the blood banking system (e.g. seasonal variation in donation) and reduce costs for this therapy.

It is important to note that, for the same clinical indication, the ideal red cell substitute for military use would also be ideal for use by civilian medical communities worldwide. Any failure of a red cell substitute to meet ideal requirements would limit its potential for use in both circumstances, military and civilian.



CURRENT TRANSFUSION RISK / UNIT

ARTIFICIAL
BLOOD

Fever, chills, urticaria	1 / 100
Hepatitis B	< 1 / 200,000
Non-A non-B hepatitis	3 / 10,000
Hemolytic transfusion reactions	1 / 6,000
Fatal hemolytic reactions	1 / 100,000
HIV	1 / 225,000*

*** (approximate U.S. average – regional variation)**

Transfusion of red cells, like all other forms of therapy, is associated with risks. The principal risks are hemolytic transfusion reactions and infection of the recipient with agents of infectious diseases transmitted by donor blood. The magnitude of these risks vary significantly from about 1/100 (one adverse effect per 100 units of blood transfused) for developing fever, chills, or urticaria (hives) to 1/100,000 for dying from a fatal hemolytic transfusion reaction.

Risks of infection of recipients with transfusion-transmitted viruses has been reduced considerably with the introduction of new blood donor screening tests. Nevertheless, small but significant risks of infection remain. The current annual risk for a recipient being infected with hepatitis B virus as a result of blood transfusion is less than 1/200,000 units transfused. The risk of non-A non-B hepatitis is approximately 3/10,000 units, with 90 percent of these cases caused by hepatitis C virus. Since new antibody tests for hepatitis C are being applied and improved, the incidence of this disease is decreasing as infected units are screened out of the blood supply.

The Centers for Disease Control and Red Cross estimates for the risk of infection with human immunodeficiency virus (HIV) from transfused blood is 1/225,000

nationwide. Risk will vary geographically based on the prevalence of HIV in the donor population.

It should be noted that these risks multiply with an increase in the number of units transfused.



HISTORY OF RED CELL SUBSTITUTES

ARTIFICIAL BLOOD

- **HEMOGLOBIN**
 - 1916 – Hemoglobin Infusion
 - 1940 – First Large Scale Transfusion Trial - USN
- **ARTIFICIAL RED CELLS**
 - 1957 – First Prepared
 - 1980s – Liposome Technology
- **PERFLUOROCARBON COMPOUNDS (PFC)**
 - 1970s – PFC Emulsions Developed
- **MODIFIED HEMOGLOBINS**
 - 1972 – Altered Oxygen Affinity
 - 1983 – Polymerized
 - 1989 – Recombinant
- **PRESENT STATUS**
 - 1984 – Military Blood Program 2004 Study
 - 1990 – FDA/NIH/DoD - "Points to Consider"
 - 1992 – Scientific Progress from Private Sector
 - Significant Unresolved Issues of Toxicity

A blood substitute has been desired since the description of the circulation of blood by Harvey in the early 17th century. Since that time, considerable work in the area of blood transfusion medicine has concentrated on solving the imposing problems of isoagglutination, coagulation, and infection, thereby greatly decreasing the dangers inherent in transfusion of blood from one person to another.

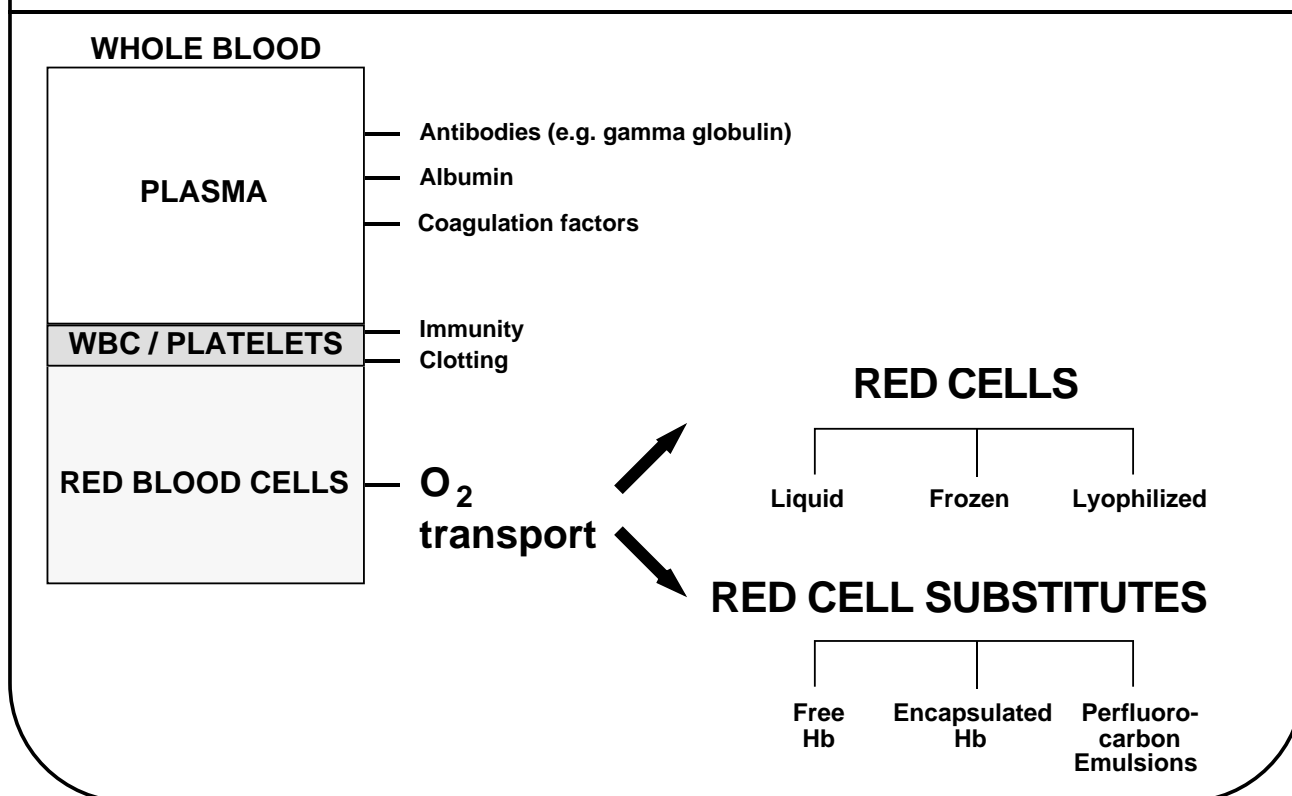
Prior to the outbreak of WWII, the National Research Council recommended establishment of a blood banking system. This system eventually made blood available on the battlefield and developed into the system of blood banks which operates today.

After the establishment of this blood banking system, improvements were continually sought. Shelf-life of stored blood was extended, and blood was successfully frozen for long-term storage. However, the search for a blood substitute was never abandoned.

Cell-free hemoglobin was used as an experimental blood substitute in the 1940s. Artificial red cells were first synthesized in the 1950s, and perfluorocarbons were developed in the 1960s. Throughout this time, much of the work was done in laboratories supported by the DoD.

In the 1970s and 1980s, hemoglobin-based products came closer to clinical use when purification and chemical modifications were thoroughly studied and understood. During these critical decades, industry became more interested in commercializing products, and a number of new companies were founded with a goal of developing and marketing a red cell substitute. In 1984, the DoD published a report (Military Blood Program 2004) which recommended continued work to improve blood storage and to develop a blood substitute.

Today, an array of products is under study and development. These include cell-free hemoglobin, encapsulated hemoglobin, and perfluorocarbon emulsions. In 1990, the FDA, DoD, and NIH, in a joint statement, recognized both the significance of further blood substitute development and the sizable obstacles that remained.



Whole blood is a tissue composed of cellular elements suspended in plasma. The cellular elements, which normally comprise about 45 percent of the volume of blood, are red blood cells, white blood cells and platelets. Plasma comprises the other 55 per cent of blood. This schematic displays the physical separation of the various components of whole blood following centrifugation. Because red blood cells are the most dense component, they descend to the bottom of the container. A very thin layer of white blood cells and platelets, collectively referred to as the “buffy coat,” rests on top of the red blood cells. Plasma is the least dense component and separates above the buffy coat.

Blood components are responsible for performing many functions. Plasma transports water and nutrients obtained from food to all cells of the body and carries waste products to the kidneys for excretion. Plasma also contains large numbers of different proteins which are vital in maintaining health. Examples of such proteins include antibodies to neutralize microorganisms and toxins, coagulation factors which are essential for blood to clot following injury to blood vessels, and albumin which helps maintain proper fluid balance within the body. There are hundreds of other types of molecules in plasma with different functions important in maintaining health.

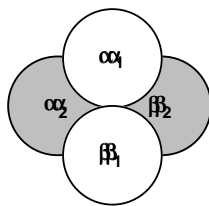
There are several types of white blood cells, or leukocytes, which help protect the body against infection and disease. Some leukocytes engulf and destroy invading bacteria, and others are important in various additional aspects of specific immunity to disease. Platelets help prevent the loss of blood from damaged blood vessels by aggregating at sites of injury to form a temporary plug or seal. At the same time, platelets release substances that start the process of blood clotting. Blood clotting factors in the plasma then form a more solid clot. Red blood cells (erythrocytes) are the most numerous of the cellular elements. Their main function is to carry oxygen from the lungs to body tissues and to carry carbon dioxide from body tissues to the lungs where it is exhaled.

In blood transfusion therapy, red cells are separated from donated blood and, after determining compatibility, infused into a patient. In addition, platelets and white cells are separated and transfused, when indicated, to treat bleeding disorders or infections, respectively. Proteins involved in coagulation, such as anti-hemophilic factors, are isolated from plasma, subjected to special fractionation procedures and administered when needed in purified form. Also, the proteins albumin and gamma globulin are fractionated from blood in a similar manner. A red cell substitute will reduce or eliminate the need for infusion of red cells; however, other cellular and plasma components of whole blood will still be needed.

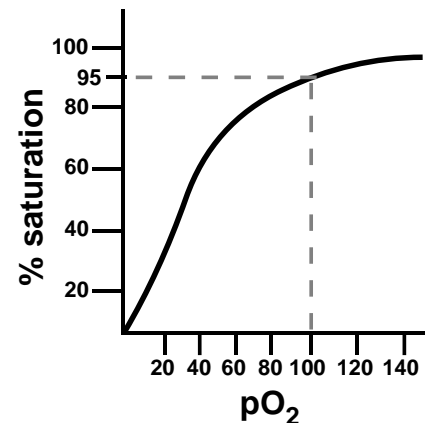
Whole blood or packed red blood cells are the only products currently available for improving oxygen transport in patients. Red cells can be stored in the liquid state for 42 days or in the frozen state for up to ten years. A lyophilized or freeze-dried red cell product is not yet available, but recent investigations have been promising enough to suggest that such a product may be available in the future.

Three major types of red cell substitutes are currently under development. Modified cell-free hemoglobin (Hb) is being investigated as a potential product because of its excellent oxygen-carrying capacity and oncotic properties. The microencapsulation of hemoglobin in lipid vesicles, which more closely resembles the arrangement seen in red blood cells, is another approach being pursued. A different approach involves the development of perfluorocarbon emulsions which have the ability to dissolve large quantities of oxygen.

- **Unique Oxygen Carrier**
- **Tetrameric Structure Required for Normal Function**
- **Even Minor Modifications Can Alter Normal Function**



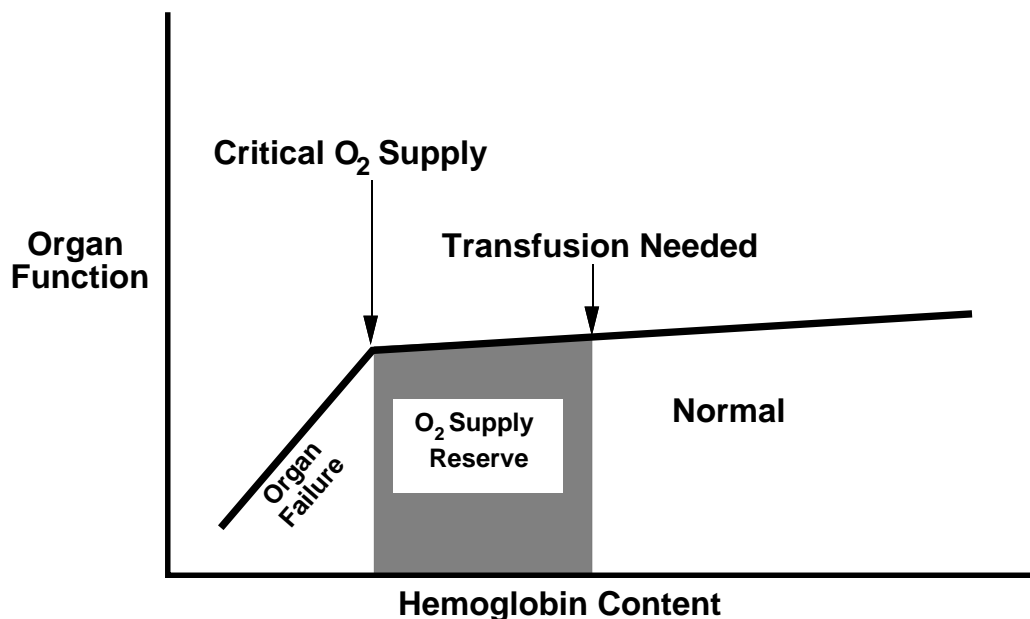
**TETRAMERIC (4 SUBUNITS)
STRUCTURE OF HEMOGLOBIN**



OXYGEN DISSOCIATION CURVE

Hemoglobin is composed of 4 subunits (2 α and 2 β), each of which carries a heme group which can bind 1 molecule of oxygen (O₂). As an O₂ molecule is bound, the affinity of the remaining binding sites for O₂ increases. This leads to the cooperative (“S-shaped”) O₂ binding curve. The molecular mechanisms which underlie these reactions are exquisitely complex and even small chemical modifications of hemoglobin affect cooperativity. Hemoglobin in the blood is 95 percent saturated (bound) with O₂ at the O₂ concentration found in room air. Carbon dioxide (CO₂) is carried in the blood physically dissolved as bicarbonate and chemically bound to hemoglobin. Some modifications of hemoglobin will alter O₂ or CO₂ binding, but there may also be other physiological and clinical consequences as yet unknown.

Oxygen molecules will bind to the iron atoms of heme when these atoms are in the reduced (Fe²⁺) state. Unfortunately, when hemoglobin is outside the red blood cells, the iron in hemoglobin is quickly converted to the oxidized (Fe³⁺) state, forming methemoglobin which no longer binds oxygen. This conversion to methemoglobin is prevented inside the red cell by a metabolic system designed to maintain iron in its reduced state.



Blood supplies O₂ to tissue. The total amount of O₂ delivered to any tissue is the product of the O₂ contained in the blood and the rate of blood flow, or cardiac output. Therefore, decreases in cardiac output, as well as decreases in hemoglobin concentration, can impair tissue oxygenation.

This figure demonstrates that as the concentration of hemoglobin (and therefore the delivery of O₂) to tissue decreases, natural compensatory mechanisms such as vasodilation and increases in blood flow preserve the function of the organ over a broad range. However, at some point, compensatory mechanisms are overwhelmed, and the supply of oxygen becomes inadequate to maintain the functions of the organ (critical O₂ supply). In the case of the brain and heart, the effects of significant oxygen deficiency are disastrous.

Ideally, the delivery of O₂ should never be allowed to reach the point of critical O₂ supply. As the figure shows, an "O₂ supply reserve" can be maintained by transfusion after blood loss, for example, during surgery or after trauma. Each organ and tissue may have a different critical O₂ supply, whereby a supply that is adequate for the liver might be inadequate for the brain. A second complicating factor is that there is no clinical measurement that can be used to assure that there is an adequate

reserve; physicians usually use the blood hemoglobin concentration or the percentage of red cells in the blood (hematocrit) as a guide for transfusion requirements. Third, it is likely that the actual critical O₂ supply may vary from patient to patient, depending on many factors such as age, state of health, physical conditioning, etc.

In today's climate of awareness of the risks of disease transmission, many patients are fearful of receiving donor blood. It is possible, then, that in many patients undergoing elective surgery, their O₂ supply reserve is small, and organ function could be endangered.



FUNCTION OF RED BLOOD CELLS

ARTIFICIAL
BLOOD

ENCAPSULATES HEMOGLOBIN:

- **Permits High Hemoglobin Concentration**
- **Maintains Hemoglobin in a Functional State**
- **Permits Useful Lifespan for Hemoglobin**
- **Protects Body from Hemoglobin and Hemoglobin from Body**

Free in solution, hemoglobin at a concentration of 15 g/dl would be so viscous that it would scarcely circulate. It would also exert an increased colloid osmotic pressure (oncotic pressure) where water would be drawn from the interstitial and intracellular spaces into blood vessels, leading to vascular congestion. Both of these problems are solved by hemoglobin being contained in a red cell. Hemoglobin concentration in the cell is approximately 32-34 g/dl. Since red cells normally occupy about half of the blood volume, the concentration of hemoglobin in the blood is about 14-15 g/dl. Blood viscosity is low because red cells are streamlined and deformable. Red cells exert no oncotic pressure, so transfusion with red blood cells poses no danger of fluid shifts. Packaging of hemoglobin within the red cells prolongs the oxygen carrying capability of molecules from a few hours to 120 days.

Another way cell-free hemoglobin can leave the circulation is by “extravasation.” That is, it crosses the endothelial lining of small vessels and enters the interstitial spaces. When this occurs, it can have several deleterious effects including reacting with endothelium-derived relaxing factor (EDRF), which is now known to be nitric oxide, and production of toxic O₂ radicals. Red cells, therefore, serve not only to protect hemoglobin from degradative mechanisms, but also to protect the body from the potentially toxic effects of hemoglobin.

V. TECHNOLOGY OVERVIEW



CURRENT APPROACHES

ARTIFICIAL BLOOD

<u>TECHNOLOGY</u>	<u>SOURCES</u>	<u>MODIFICATIONS</u>	<u>PRODUCT</u>
Natural/Purified Hemoglobin	Human Non-Human	Chemical: <ul style="list-style-type: none">• Cross-linking• Polymerization• Conjugation Encapsulation	Modified Human or Non-Human Hemoglobin
Recombinant Hemoglobin	Human Hb in: <ul style="list-style-type: none">• Bacteria• Yeast• Transgenic Animals	Genetic: <ul style="list-style-type: none">• Cross-linking• Polymerization• Conjugation Encapsulation	Modified Human Hb
Chemical Synthesis	Manufacturing	Emulsion	PFC Emulsions

All of the products used for red cell replacement can be categorized by the technology used in manufacture. Hemoglobin can be purified from either human or animal blood and used as a “stroma-free” product for blood replacement. The most common animal hemoglobin used has been bovine.

Hemoglobin can also be produced by recombinant technology in bacteria, yeast, or transgenic animals. Because the gene sequences used in this technology originate from the human gene sequence, the product is considered human hemoglobin purified from the bacterial or animal cells which have been used to produce it.

Before hemoglobin from any source can be used as a blood substitute, it is modified by chemical cross-linking, polymerization, conjugation to macromolecules, or encapsulation in liposomes. Recombinant technology is advantageous in this respect because some of these modifications can be engineered into the hemoglobin molecule by alteration of the gene. This would be expected to result in subsequent production of consistent product.

Red cell substitutes may also be chemically synthesized. Perfluorocarbons are an example of this type of product.

- **Cross-linked**
- **Polymerized**
- **Conjugated**
- **Genetically Engineered**
- **Encapsulated**

Research in developing a hemoglobin-based red cell substitute has progressed in three areas. These are chemical modification (including cross-linking, polymerization, and conjugation), genetic engineering, and encapsulation in liposomes. These modifications are designed to help overcome the limitations associated with stroma-free hemoglobin.

The following definitions distinguish these modifications:

Cross-linked:	Hemoglobin stabilized in the tetramic structure by an internal cross-link. Molecular weight 64,000.
Polymerized:	Aggregates of hemoglobin tetramers linked covalently. Molecular weight greater than 64,000.
Conjugated:	Hemoglobin tetramers chemically linked to dextran or other macromolecules. Molecular weight variable but greater than 64,000.

Genetically engineered:	Hemoglobin produced in a non-human organism such as bacteria, yeast, or transgenic animals. Structure and molecular weight are determined by the DNA sequence.
Encapsulated:	Any hemoglobin product contained within a synthetic vesicle or membrane.



EFFECTS OF STROMA-FREE (UNMODIFIED) HEMOGLOBIN

ARTIFICIAL
BLOOD

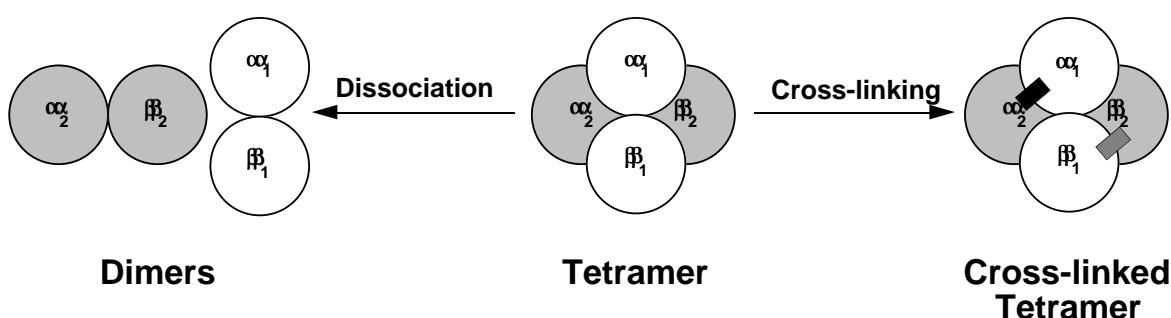
<u>PROPERTY</u>	<u>EFFECT</u>
High O ₂ Affinity	Reduced O ₂ Release
Excretion by Kidney	Renal Failure
Extravasation	Vasoconstriction
High Oncotic Pressure	Vascular Congestion

Hemoglobin that has been freed from the red cell membrane (stroma-free hemoglobin) can carry O₂. Outside the red cell, however, hemoglobin loses its tetrameric structure and dissociates into dimers.

Increased O₂ affinity of stroma-free hemoglobin results in reduced release of O₂ in tissues. Excretion of free hemoglobin by the kidneys results in rapid removal from the circulation, damage to renal tubules, and ultimately, renal failure. Extravasation of free hemoglobin from the circulation probably explains vasoconstriction. Finally, the high oncotic pressure of stroma-free hemoglobin results in significant vascular congestion.

When hemoglobin dimers leave the vascular space, they can be found in nearly all tissues in the body. Therefore, organ-specific toxic effects would be both expected and unpredictable.

- Increase Persistence in the Circulation
- Decrease Oncotic Pressure
- Modify Oxygen Affinity



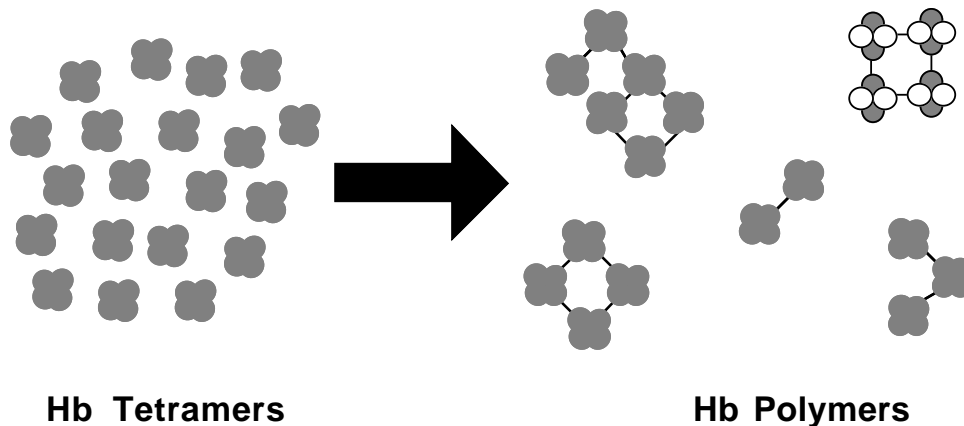
Definition: Hemoglobin stabilized in the tetrameric structure by an internal cross-link. Molecular weight 64,000.

In more dilute solution than that found in red cells, the tetrameric hemoglobin molecule tends to spontaneously dissociate. This is undesirable because the smaller molecular weight dimers and monomers are rapidly lost from the circulation. This loss of hemoglobin from the circulation may be prevented by chemical cross-linking of the tetrameric form of the molecule. Cross-linking helps maintain the “normal” tetrameric structure and molecular size of hemoglobin. This will not only reduce the loss (or increase the retention) of hemoglobin in the circulation, but will also prevent or reduce the exposure of renal tubules to hemoglobin. Cross-linking of this molecule also prevents rise in oncotic pressure induced by the spontaneous dissociation of the molecules and subsequent dilution and fluid flux into the vascular space.

There are several unresolved questions which can only be answered by further research. The first is whether the intramolecularly cross-linked hemoglobin is antigenic in the large doses that must be administered therapeutically. If so, subsequent administration of a similar product may cause an “allergic” reaction. The second is the *in vivo* stability, or rate of breakdown of the cross-link, and the effect of

hemoglobin breakdown products liberated in the circulation. The third question needing resolution is whether the optimal cross-linking procedure has been found and by what method the products of incomplete cross-linking need to be removed. Lastly, the required standards of “purity” and composition will need to be rigorously defined.

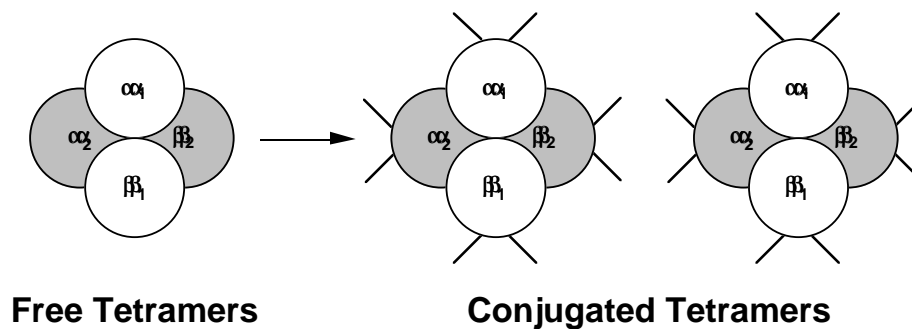
- **Permits Higher Concentration of Hemoglobin in the Blood**
- **Increases Persistence in the Circulation**

POLYMERIZATION

Definition: Aggregates of hemoglobin tetramers linked covalently. Molecular weight greater than 64,000.

Chemical linkers have been developed to attach multiple hemoglobin tetramers together by stable chemical bonding. The reaction is difficult to control and yields various sizes of polymers. Because the resulting larger molecules are retained significantly longer in the circulation, this process has been adopted by a number of manufacturers as their modification of choice. Moreover, poly-hemoglobin permits the maintenance of a higher total hemoglobin concentration in the plasma, without unacceptable fluid shifts into the circulation.

- **Linked to Biocompatible Polymers**
- **Prolongs Persistence in the Circulation**



Definition: Hemoglobin tetramers chemically linked to dextran or other macromolecules. Molecular weight variable but greater than 64,000.

Another strategy to stabilize hemoglobin in the circulation is to join it chemically to polymers such as dextran or polyethylene glycol.

While such conjugation is feasible, the biological properties of the conjugated material will have to be carefully studied with regard to compatibility, toxicity, and antigenicity.



GENETICALLY ENGINEERED HEMOGLOBIN

ARTIFICIAL
BLOOD

- **Achieves Same Objectives as Chemical Modifications**
- **Homogeneous Product**
- **Potentially Unlimited Supply**

Definition: Hemoglobin produced in a non-human organism such as bacteria, yeast or transgenic animals. The structure and molecular weight are determined by the genes.

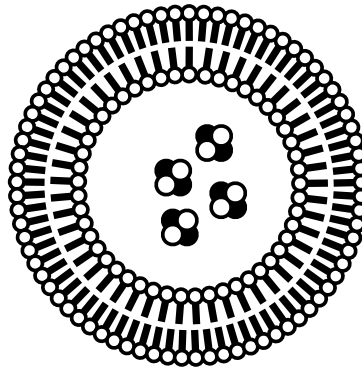
Genetically engineered hemoglobin can be produced as a recombinant protein product and achieve the same objectives as chemical modification of hemoglobin. This has been an area of active interest, and it is now possible to produce both alpha and beta chains of human hemoglobin in bacteria, yeast, or transgenic animals. The recombinant approach has particular promise because it is possible both to construct and produce either naturally occurring or novel variants of hemoglobin that differ in cross-linking or O₂ binding affinity.

The advantages of producing hemoglobin as a recombinant product include the possibility of producing large amounts of a pure product as well as the ease and reliability of production. However, there are some potential disadvantages. Use of a large amount of product in vivo would make low level contamination with bioactive molecules unacceptable. Hemoglobin produced in bacterial expression systems may be contaminated with bacterial products, including endotoxin, which may be difficult to separate. Endotoxin contamination may be minimized by use of yeast expression

systems. Production of quantities of recombinant hemoglobin required for eventual clinical use has not been demonstrated. Issues such as cost, environmental impact, and the complexity of purification procedures are not yet resolved.

Human hemoglobin genes have also been engineered into transgenic mammals. This non-human blood source can be used to produce large quantities of human hemoglobin, but problems of purification remain. These purification problems are complicated by the potential immunogenicity of animal hemoglobin and other animal products. It is also unknown whether transmission of animal disease to humans will be a problem.

- **Protects Body from Hemoglobin**
- **Protects Hemoglobin from Body**
- **Minimizes Oncotic Activity**
- **Prolongs Intravascular Persistence**



**Liposome - Encapsulated
Hemoglobin**

Definition: Any hemoglobin product contained within a synthetic vesicle or membrane.

The membrane of the normal red blood cell separates the hemoglobin contained in the interior of the cell from the other components of blood and from other cells in the body. Red blood cell membranes, in addition to expressing the blood group antigens, are complex structures composed mainly of various lipids. All such membranes also contain proteins, carbohydrates, and various channels through which chemicals can move into and out of the cell interior. The interior of the red blood cell is a unique environment precisely adapted to maximize the oxygen carrying capacity of hemoglobin.

Encapsulation offers protection from the inherent toxicity of hemoglobin and increases persistence of hemoglobin in the circulation. Several groups have produced suspensions of hemoglobin encapsulated into small liposomes that are intended to function as biocompatible artificial red cells without blood group antigens. Oxygen freely diffuses across the membrane and the half-life of hemoglobin contained in the liposome circulating in the vascular space is prolonged. The half-life is still much shorter than that of hemoglobin in normal red cells.

Potential problems with the production of liposome-encapsulated hemoglobin (LEH) include: the relatively poor efficiency of hemoglobin incorporation into the liposome; the physical instability of liposomes in storage leading to size rearrangement and hemoglobin loss; the chemical instability of the lipid-hemoglobin interface leading to lipid peroxidation and hemoglobin denaturation; and possible contamination with bacterial endotoxin.

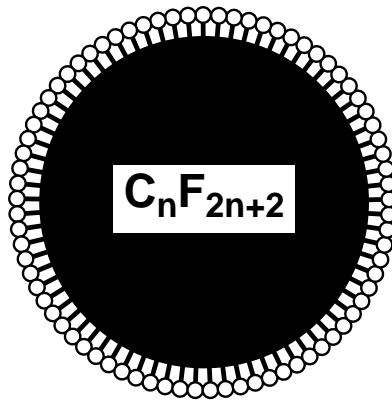
Physiological problems with liposome-encapsulated hemoglobin include low oxygen-carrying capacity, increased blood viscosity, and reticuloendothelial blockade. Systematic development of liposome-encapsulated hemoglobin technology is dependent on the consistent availability of high quality hemoglobin and the scale-up of liposome production.



PERFLUOROCARBON PRODUCTS

ARTIFICIAL
BLOOD

- Chemically Inert
- No Disease Transmission
- Easy to Manufacture



PFC "Particle"

Definition: Highly fluorinated hydrocarbons that are liquid at body temperature and are excellent solvents for oxygen and other gases.

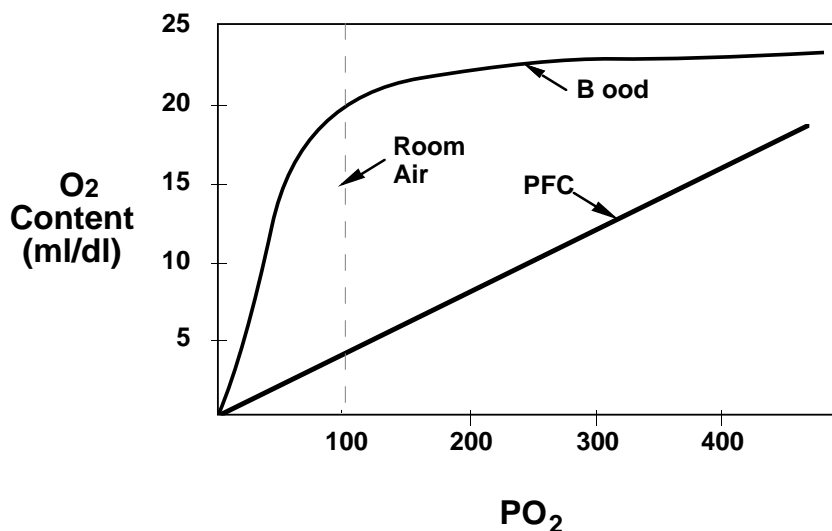
The prototype of the first generation product was formulated using two perfluorocarbon (PFC) compounds and a commercially available emulsion stabilizer. Its PFC content was only 11 percent by volume which was too low for efficacy as a red cell substitute. It was stored as three components, one of which was frozen and required reconstitution before use. After extensive testing, that product could not be approved as a red cell substitute, but was later approved for intracoronary injection in small doses during percutaneous transluminal coronary angioplasty.

The prototype of the second generation product is formulated using a bromine-containing perfluorocarbon, emulsified with a lecithin-based emulsifier. It contains about four times as much PFC as the first generation product, and therefore, can carry four times as much oxygen. It can be stored at room temperature and does not require reconstitution before use.

Because of the synthetic nature of PFC preparations, the potential for transmission of human disease is nearly eliminated. Because of the chemical inertness of these

compounds, biodegradation or the generation of toxic or antigenic products seems unlikely. The relatively simple manufacturing processes involved in the production of commercial quantities of product may result in relatively low product cost.

- **Dissolves (does not bind) Oxygen**



In contrast to hemoglobin, which binds oxygen chemically and is nearly completely saturated when breathing room air, perfluorocarbons have a high “solubility” for oxygen. As a result, the higher the concentration of oxygen inspired, the more oxygen will be dissolved. Thus, significant clinical benefit requires the use of supplemental oxygen. This will likely present logistical limitations outside the hospital setting.

While the chemical inertness of PFC compounds is a desirable property, they do not mix with water and can not dissolve vital constituents (e.g. salts) essential to permit intravenous administration. To administer PFCs intravenously, they must be prepared as an emulsion, consisting of small (0.1-0.3mm) “droplets” of PFC suspended in an aqueous medium. These emulsions must also be stabilized by additives. For maximum benefit, a biologically usable emulsion must be formulated in such a way that the PFC content is relatively high (probably >30 percent volume/volume). Such emulsions are highly viscous, and blood-PFC mixtures of high PFC content may have impaired flow properties. Toxicities and impurities in the emulsifiers also must be investigated.

After intravenous administration, the PFC “droplets” are gradually removed from the circulation by uptake into the reticulo-endothelial system (liver, spleen, etc.) at a rate largely dependent on the total amount of PFC administered. A large load of PFC causes substantial enlargement of the organs of the reticulo-endothelial system and may impair the ability of the body to deal with infections. Most importantly, Phase I clinical trials of perfluorocarbon emulsions have demonstrated adverse clinical reactions including fever, nausea, and “flu-like” symptoms.

VI. DEVELOPMENT OF RED CELL SUBSTITUTES



- **Identify an Acceptable Product**
- **Develop Manufacturing Procedures**
- **Conduct Pre-clinical Testing**
- **Conduct Clinical Trials**
- **Establish Production Facility**
- **Obtain Regulatory Approval**

Identify an acceptable product. The first step in the development of a new drug or biologic is the definition of a “product.” In the case of a red cell substitute, this would entail physicochemical characterization of the product and a description of its preparation and major biologic properties. This definition should be such that succeeding lots of product will be equivalent and tests can be developed to ensure such consistency and identity.

Develop manufacturing procedures. In the typical situation, a product is first produced in small quantities in a research laboratory. In order to make larger quantities of promising products, the production is then scaled-up to a pilot plant and, in certain cases, to full-scale production level. In such a progression, the manufacturer attempts to maintain the functional aspects of each preparative step as the size increases, but this is not a simple matter. For example, quality control of a one hundred milliliter chromatographic column is very different from that of a five liter apparatus. All the details of the manufacturing procedure must be carefully developed before product approval. The effect(s) on the product of any subsequent changes in the procedure must be demonstrated.

Conduct pre-clinical testing. The product must be evaluated using *in vitro* and animal studies to support safety in planned human trials. In the case of hemoglobin-derived red cell substitutes, the FDA has issued *Points to Consider* which provide for the selection of appropriate animal models in an effort to approximate the intended clinical situation during pre-clinical testing.

Conduct clinical trials. The classical approach includes three phases:

Phase I: studies in humans, usually normal volunteers, to demonstrate safety of the product at doses equivalent to the intended therapeutic doses.

Phase II: studies in patients to show the desired effect(s) of the product and the dependency of the effect(s) upon dose.

Phase III: a controlled study of efficacy and safety. The pivotal study will usually be a double-blind, controlled study in which neither investigators nor patients know which patients received treatment or placebo. Careful statistical analysis is part of such studies.

Establish production facility. Plants for commercial production of the product must be established in compliance with the rigorous and expensive requirements of good manufacturing practices (GMP).

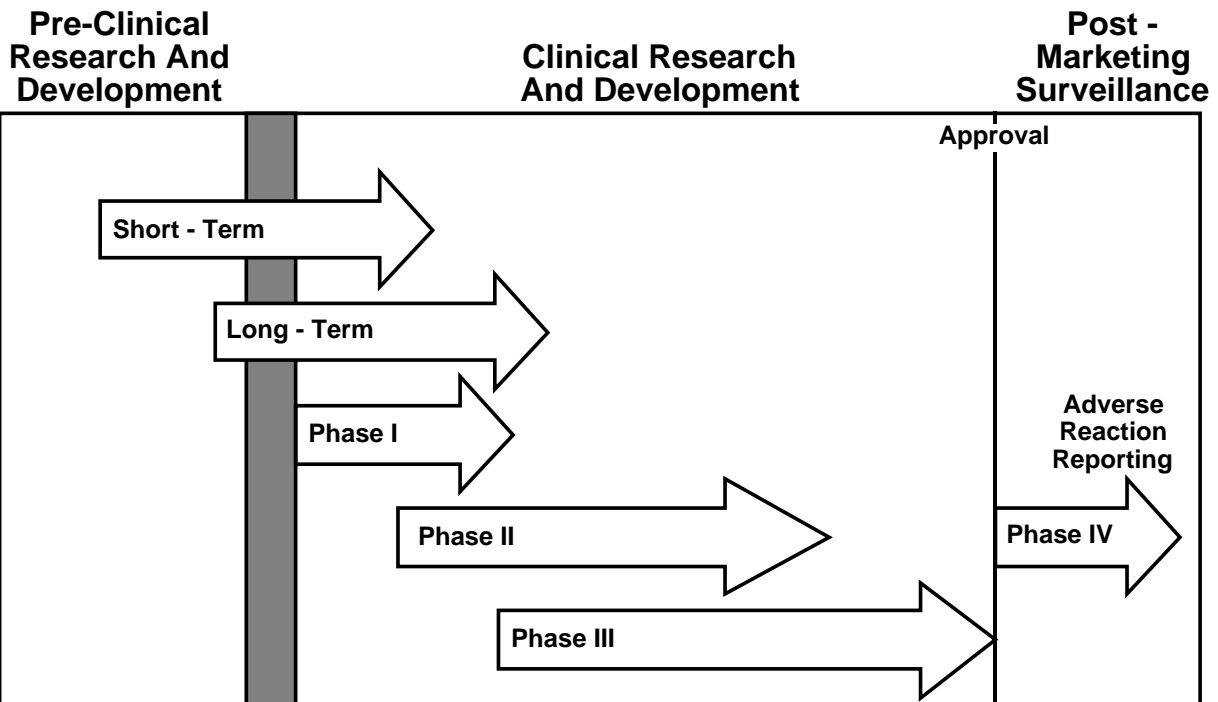
Obtain regulatory approval. A license for a biologic product is legal permission to sell, barter, or trade such product in interstate commerce. The analogous mechanism for a drug is a New Drug Application (NDA). The approval process for both includes evaluation of the product and the establishment in which it is to be produced. Supporting data are accumulated prior to application, in part through an Investigational New Drug Permit (IND) which allows study of the test agent in human subjects under FDA monitoring. The approval process also includes inspection of the manufacturing establishment. After initial approval, the safety and efficacy of the product are followed by submission of adverse reaction reports, review of lot specifications, review of quality control data, and, in some cases, by individual lot release performed by FDA. The establishment is also inspected on a regular basis.

The length and complexity of the approval procedure will vary with the quality and completeness of data supporting the application. This is usually related to the level of understanding about the product. Approval of red cell substitutes poses unique problems in this regard.



NEW DRUG DEVELOPMENT

ARTIFICIAL
BLOOD



Once a red cell substitute with a reasonable prospect for success is identified, evaluation revolves initially around issues of safety and efficacy, and proceeds from simple testing methods to those more expensive and elaborate. Generally, efficacy is the original focus.

Fairly simple *in vitro* tests establish the oxygen-loading and unloading characteristics of the product. Normally, this would involve testing in a large number of small animals that are exchange-transfused down to levels of native red cell concentrations incompatible with life. Control animals are similarly exchange-transfused but without the administration of oxygen-delivering component. If the test material can support life in the absence of sufficient red cells, more elaborate models may be tried involving various challenges such as hemorrhage. The candidate material would be compared to currently used resuscitative practices, perhaps including both blood and non-blood materials. Survival is generally the end-point of testing for efficacy in these small animal models.

If the product still shows promise, testing then proceeds to larger animals. This allows use of challenges and resuscitation protocols that more closely mimic clinical reality. The larger size of the animals also allows serial sampling of blood and serial

measurements of various pertinent physiologic parameters. End points of evaluation can be various indices of adequacy of oxygen delivery that are more subtle and sensitive than survival. Basic studies include monitoring organ function by periodic sampling of blood or physiologic monitoring, as well as by morphologic studies on harvested organs.

If the material shows a promising ratio of safety to toxicity and if other considerations such as cost and manufacturing processes are acceptable, the material and pertinent data can then be reviewed by the FDA to see if testing in humans is appropriate. If the product is safe (Phase I) and demonstrates efficacy (Phase II), it is then allowed to undergo Phase III testing. This consists of prospective, randomized trials conducted by selected designated clinical investigators using human subjects who have given informed consent. If all of this testing confirms that the product is useful, it can then be marketed, but evaluation continues in the form of reporting of adverse reactions to the manufacturer and to the FDA. Particular attention is paid to products newly entering the market place. This post-marketing surveillance is designated Phase IV.

- **Phase I Demonstration of Product Safety**
- **Phase II Demonstration of Product Efficacy**
- **Phase III Controlled Study of Safety & Efficacy**

Regulatory approval of products intended to serve as red cell substitutes will require demonstration of clinical efficacy. Such demonstration can be accomplished by means of a clinical trial (an experimental study performed on human subjects who have provided informed consent), organized to obtain the needed data and ensure the safety of the study subjects.

The classical clinical trial approach can be subdivided into three phases:

Phase I: studies in humans, usually normal volunteers, to demonstrate safety of the product at doses equivalent to the intended therapeutic doses.

Careful observation of study subjects can lead to Phase II clinical trials, to modification of a product, or to discontinuation of the development effort.

Several red cell substitutes have been subjected to Phase I trials in which a number of adverse reactions were observed.

Phase II: studies in patients to show the desired effect(s) of the product and the dependency of the effect(s) upon dose.

No products currently under development have reached Phase II studies. Such trials would require demonstration that the product carries oxygen and that the oxygen transported has a beneficial effect on a human system.

A trial with red cell substitutes might show that the oxygen delivery of circulating blood has been enhanced in the presence of the test product and that the metabolism of exercised muscles perfused with the product is improved as compared with a control.

Phase III: a controlled study of efficacy and safety. The pivotal study will usually be a double-blind, controlled study in which neither investigators nor patients know which patients received treatment or placebo. Careful statistical analysis is part of such studies.

Such studies must demonstrate a clinical benefit deriving from use of the product. The definition of benefit in terms of study endpoints has not been established for red cell substitute products.

The selection of criteria for efficacy in the case of red cell substitutes will depend upon the indication chosen. Since this phase of the study must be controlled, subjects are randomly assigned to a treatment or a control (placebo) group. It is in the design of these studies that risks and benefits of any proposed product must be carefully considered.



STATE OF TESTING OF RED CELL SUBSTITUTES IN HUMANS

ARTIFICIAL
BLOOD

- **Only Preliminary Data are Available**
- **Manufacturers are Reluctant to Reveal Adverse Medical Events**
- **Lack of Understanding of Adverse Reactions is Inhibiting Further Progress**
- **Adverse Reactions Reported to FDA in Some Patients Include:**

Fever

Headache

Abdominal Pain

Muscle Aches

Increased Blood Pressure

Decreased Heart Rate

Chest Pain

Evidence of Liver Dysfunction

Preclinical studies suggested that the risk/benefit ratio was favorable for a number of products, and Phase I clinical trials in humans appeared to be justified. These products included both hemoglobin-based and perfluorocarbon-based red cell substitutes.

In general, manufacturers presented the Panel with important information about specific product characterization and the nature of product modifications. Unfortunately, most manufacturers were less candid about critical information regarding toxicities observed in animal studies and even less forthcoming with detailed information about clinical findings in humans. This reluctance to release clinical data in early phases of drug development is not unique.

Since this expert Panel was mandated by Congress to provide guidance and assess the status of current technologies, it was critical that the preliminary data from Phase I clinical trials be made available. To this end, FDA participants on the Panel were authorized to provide a summary of adverse reactions. Such information did not include proprietary manufacturing data.

Adverse reactions reported in Phase I trials in humans were not predicted by the prior animal studies. The doses of product were small and were given to healthy, well-hydrated, normal individuals. Reactions in some volunteers included fever, headache, abdominal pains, muscle aches, increased blood pressure, decreased heart rate, chest pain, and evidence of liver dysfunction.

Not all products produced all reactions. No product to date, however, has been devoid of adverse findings in Phase I trials reported to the FDA, and no product has been allowed to progress to Phase II trials for this reason.



ACCOMPLISHMENTS OF PRIVATE SECTOR INVESTMENT

ARTIFICIAL
BLOOD

- **Pilot Manufacturing Facilities**
 - **Purification**
 - **Polymerization**
 - **Genetic Modification**
 - **Perfluorocarbon Emulsions**
- **Attracting Investment Capital**
- **Applied Research**
- **Large Amount of Proprietary Information Generated**

During the past ten years, using the basic scientific research information that was produced through funding by the NIH and DoD, the private sector has developed pilot facilities to produce hemoglobin-based and perfluorocarbon-based oxygen carriers.

Human hemoglobin, bovine hemoglobin, and genetically engineered recombinant human hemoglobin are now being produced. Plants in compliance with good manufacturing practices (GMP) are being established by private companies to produce purified, polymerized, conjugated, genetically modified encapsulated, and non-encapsulated human and non-human hemoglobin, as well as perfluorocarbon emulsions. Testing of red cell substitutes in animals and in clinical trials to assess safety in normal volunteers is in progress. A large amount of proprietary information has been collected by industry.

VII. CONCLUSIONS AND RECOMMENDATIONS

- **A red cell substitute is both needed and feasible**
- **Major advances have occurred in product development**
- **Safety in humans remains a major concern**
- **Much of the enabling basic research has resulted from Federal funding**
- **Recent development has been funded and conducted primarily by industry**
- **All clinical testing is in preliminary stages**

The Panel on Delivery of “Artificial Blood” to the Military was made up of representatives from industry, academia, DoD, NIH, and the FDA, representing expertise in trauma medicine, blood banking, hematology, physiology, and pharmacology. Presentations were made by 11 private companies, as well as by individuals from universities, research groups, and the Department of Defense. Detailed descriptions of many different products currently under development were presented, studied, and discussed in detail. The stage of development of these products varied from concept only to those that are in Phase I clinical trials in humans. In addition to the presentations from invited experts and company representatives, the Panel had access to voluminous published literature and information made available by the FDA concerning clinical trials.

After more than a century of research and an enormous investment of public funds in research, the conclusion reached over the past decade was that red cell substitutes could be developed successfully. Subsequently, considerable progress has been made by industry in modification (both chemical and genetic) of hemoglobin, artificial encapsulation of hemoglobin, and formulation of perfluorocarbon emulsions, and very sophisticated products have been developed for animal testing. **Nevertheless, products that apparently are safe in animals have produced unpredicted side effects in human clinical trials.**

- **Unexpected adverse events and the inherent complexity of development preclude regulatory approval within several years**
- **The field is not sufficiently advanced to identify a leading technology or product**
- **Progress in the field now appears to be stalled because of a lack of pertinent fundamental knowledge**
- **Results of proprietary industrial research are generally not available to the scientific community**

The side effects noted in human trials are not well understood and have not been reported in the peer-reviewed scientific literature. Based on the limited information available, however, it appears that the adverse effects are of a general nature and affect multiple organ systems. The complexity of the toxicity problem suggests that it is unlikely that a product will be approved for human use in the next several years. Too little is known about too few products to be able to identify a leading candidate.

The bulk of research and development in red cell substitutes is currently being conducted in industrial laboratories because only industry has the facilities to produce products with sufficient purity and quality control to perform biological studies in animals and other *in vivo* systems. Such research often does not benefit from peer-review and scrutiny in the scientific literature and is often not disseminated widely in the scientific community. This research will not have the wide impact that publicly funded, peer-reviewed, published research would have.

The Panel recognizes the desire of Congress for specific detailed recommendations for development of red cell substitutes. However, since the field is not sufficiently advanced to identify a leading technology at this time, the Panel believes the National interest will be best served by supporting necessary enabling research in this field, rather than by funding product development.

- **Congress should commit to a National program in red cell substitutes that will accomplish the following:**
 - **Establish a specific peer-reviewed funding mechanism to support red cell substitute research**
 - **Expand the critical mass of investigators**
- **DoD and NIH, with the active support of the FDA, should be directed to work together to:**
 - **Procure sources of suitable representative research material**
 - **Make these materials available to approved investigators based on scientific merit**

The tasking requested that the Panel recommend actions necessary to accelerate the availability of artificial blood substitute products through testing, development of manufacturing methods or other developmental support, and to develop a plan that identifies a development framework, timetable, and investment recommendation for the Congress.

The Panel concluded that, for any product, significant additional basic research and pre-clinical testing are required before moving into advanced clinical trials or final product development and manufacturing.

The primary recommendation of this Panel is that in order to attempt to achieve a red cell substitute available to the military and the general population within a five-year time frame, Congress should commit to a National blood substitutes program beginning in FY 93.

The Congress should direct the DoD and the NIH, with the active support of the FDA, to work closely together to: establish a basic and applied research program based on scientific merit and peer review; procure sources of red cell substitute materials for this research; and increase the number of investigators in this field.

- **Congress should appropriate \$50 million per year for five years for basic research**
- **Experimental results derived from government funding must remain in the public domain; i.e. published in the open scientific literature**
- **Large-scale product development and manufacturing should be done by industry**

All experimental results derived from government funding must be disseminated in the peer-reviewed scientific literature in a timely manner, or otherwise remain in the public domain.

Funding for product development and manufacturing should continue to come from industry, while basic research and development and associated testing should be funded by a government sponsored National red cell substitute program. In order to initiate this program, Congress should appropriate \$50 million per year in FY 93, 94, 95, 96, and 97 for a combined DoD/NIH red cell substitute research program. This funding should not be to the exclusion of other blood related research.

The research program investigators should be tasked to address the issues which the Panel found to be major obstacles to implementation. Some examples of appropriate research topics are:

- Provision of standardized sources of hemoglobin for research.
- Studies of the interaction of hemoglobin (or perfluorocarbon emulsions) with endothelium and macrophages.

- Development of animal models that simulate clinical use, such as trauma, shock, infection, and surgical blood loss.
- Studies relating to the biochemical modification of hemoglobin with its biological effect.
- Studies of encapsulation of hemoglobin (or perfluorocarbons) into artificial vesicles and their biochemical, physical, physiological, and biological effect.
- Studies of the tissue distribution and metabolic fate of modified hemoglobins, artificial vesicles, and perfluorocarbon emulsions.

VIII. APPENDIX A – GLOSSARY

I. TECHNICAL

CO ₂ –	Carbon Dioxide
EDRF –	Endothelium-Derived Relaxing Factor
FDA –	Food and Drug Administration
GMP –	Good Manufacturing Practices
Hb –	Hemoglobin
HIV –	Human Immunodeficiency Viruses
IND –	Investigational New Drug Permit
LEH –	Liposome-Encapsulated Hemoglobin
NDA –	New Drug Application
O ₂ –	Oxygen
PFC –	Perfluorocarbon

II. ORGANIZATIONAL

ASN(RDA) –	Assistant Secretary of the Navy (Research Development and Acquisition)
DoD –	Department of Defense
DoN –	Department of Navy
NRAC –	Naval Research Advisory Committee
OCNR –	Office of the Chief of Naval Research
OSD –	Office of Secretary of Defense

IX. APPENDIX B – ADDITIONAL RESOURCE MATERIAL

1. Biro, G. P. and P. Blais. 1987. Perfluorocarbon blood substitutes. *CRC Critical Reviews in Oncology/Hematology*. 6:311-373.
2. Carson, J. L., L. B. Russell, M.I. Taragin, F. A. Sonnenbery, A. E. Duff, and S. Bauer. 1992. The risks of blood transfusion: The relative influence of acquired immunodeficiency syndrome and non-A non-B hepatitis. *Am. J. Med.* 92:45-52.
3. Carter, T. H. 1991. Biotechnology, economics, and the business of blood. *Biotechnology*. 19:3-30.
4. Centers for Biologics Evaluation and Research. 1991. Points to consider in the safety evaluation of hemoglobin-based oxygen carriers. *Transfusion*. 31:369-371.
5. Chang, T.M.S. and C. Lister. 1990. *Biomat. Art. Cells Art. Org.* 18:693-701..
6. Goins, B., A.S. Rudolph, F. S. Ligler. 1991. Liposome - encapsulated hemoglobin: historical development of a blood substitute. *Biotechnology*. 19:117-125.
7. Gould, S. A., L. R. Sehgal, H. L. Sehgal, G. S. Moss. 1992. Artificial blood: current status of hemoglobin solutions. *Crit. Care. Clin.* 8:293-309.
8. Kaufman, R. J. 1991. Medical oxygen transport using perfluorochemicals. *Biotechnology*. 19:127-162.
9. Sheffield, C. L. and J. R. DeLoach. 1991. Non-encapsulated hemoglobin based red blood cell substitutes: An updated view. *Biotechnical. Appl. Biochem.* 14:249-255.
10. Snyder, S. R. and J. A. Walder. 1991. Chemically modified and recombinant hemoglobin blood substitutes. *Biotechnology* 19:101-116.
11. Vogel, W. Mark, R. C. Dennis, G. Cassidy, C. S. Apstein, and C. R. Valeri. 1986. Coronary constrictor effect of stroma-free hemoglobin solutions. *Am. J. Physiol.* 251:H413-H420,
12. Winslow, R. M. 1992. Hemoglobin-based red cell substitutes. Baltimore: Johns Hopkins University Press. 242 pp.

**X. APPENDIX C - DEPT OF HEALTH
AND HUMAN SERVICES LETTER OF
11 MARCH 1992 TO OFFICE OF THE
CHIEF OF NAVAL RESEARCH**



Food and Drug Administration
Rockville MD 20857

Sophie A. Krasik, Esquire
Department of the Navy
Office of the Chief of Naval Research
Arlington, Virginia 22217-5000

Re: Your number 5800, Ser OCCC/219

Dear Ms. Krasik:

This responds to your letter of March 2, 1992, requesting access to records regarding artificial blood for the purpose of a congressionally requested study by the Naval Research Advisory Committee, a Federal advisory committee of the Department of the Navy.

Pursuant to 21 CFR 20.85, I have authorized employees of the Center for Biologics Evaluation and Research to provide access to information otherwise exempt from disclosure. This authorization does not extend to trade secret information prohibited from disclosure by statute.

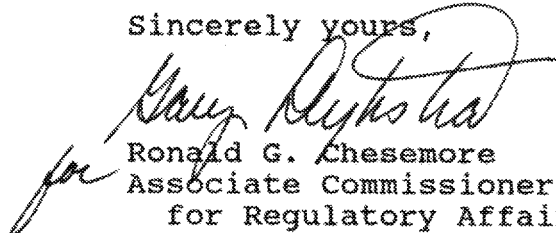
This authorization is conditioned on your taking appropriate safeguards to ensure that these records are not disclosed to individuals other than advisory committee members or Department of the Navy employees. Any other disclosure of the information can be made only with the advance written consent of the Food and Drug Administration. Your letter specifies that you will comply with these conditions.

You have asked that Dr. Cavagnaro and Dr. Fratantoni identify information that is confidential. Neither of these individuals will be able to identify all of the confidential material in advance. Identification of confidential material in advance would slow Navy's access to the records. Catherine Lorraine of our Office of General Council and members of your staff have already discussed in general terms the kinds of information that FDA considers confidential.

Page 2 - Ms. Krasik

Please feel free to contact Irene Kelly of my office if you need further assistance (301-443-1500).

Sincerely yours,


for Ronald G. Chesemore
Associate Commissioner
for Regulatory Affairs

cc: Dr. Gerald Quinnan (HFB-1) with incoming
Dr. Joseph Fratantoni (HFB-420) with incoming
Dr. Joy A. Cavagnaro (HFB-300) with incoming